compared with published experimental results. Further, we show how our novel definition of DNA-binding can be used for constructing improved machine learning classifiers for automatic identification of DNA-binding residues.

RNA Folding

2438-Pos
Probing the Dynamics of the P1 Helix within the Tetrahymena Group I Intron
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RNA conformational transformations are integral to RNA's biological functions. Further, structured RNA molecules exist as a series of dynamic intermediates in the course of folding or complexation with proteins. Thus, an understanding of RNA folding and function will require deep and incisive understanding of its dynamic behavior. However, existing tools to investigate RNA dynamics are limited. Here we introduce a powerful fluorescence polarization anisotropy approach that utilizes a rare base analog that retains substantial fluorescence when incorporated into helices. We show that 6-methylisoxanthopterin (6-MI) can be used to follow the nanosecond dynamics of individual helices. We then use 6-MI to probe the dynamics of an individual helix, referred to as P1, within the 400nt Tetrahymena group I ribozyme. Comparisons of the dynamics of the P1 helix in wild type and mutant ribozymes and in model constructs reveal a highly immobilized docked state of the P1 helix, as expected, and a relatively mobile 'open complex' or undocked state. This latter result rules out a model in which slow docking of the P1 helix into its cognate tertiary interactions arises from a stable alternatively docked conformer. The results are consistent with a model in which stacking and tertiary interactions of the A3 tether connecting the P1 helix to the body of the ribozyme limit P1 mobility and slow its docking, and this model is supported by cross-linking results. The ability to isolate the nanosecond motions of individual helices within complex RNAs and RNA/protein complexes will be valuable in distinguishing between functional models and in discerning the fundamental behavior of important biological species.

2439-Pos
Analysis of RNA Hairpin Folding by Dual Beam Fluorescence Fluctuation Spectroscopy
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Hairpin formation is one of the central problems of nucleic acid folding. Experimental and theoretical results from several laboratories indicate that in many cases formation of RNA hairpin cannot be treated as a simple two-state process. We use fluorescence fluctuation spectroscopy (FFS) to characterize folding kinetics of two RNA hairpins (CGGUUUCCCUCCCACCUUUGCCG and CGGUUUUUUUUUUUUUUUGCCG) each labeled with a fluorophore and a quencher (TAMRA and dabcyl) on the 3' and 5' end respectively. FFS is unique in its ability to measure relaxation rates at equilibrium, and coupling this technique to flow allows for simultaneous detection of the end-to-end contact and diffusion coefficient of the molecules. The two hairpins studied here have same stem but different loop sequences, and display different folding kinetics as a function of K^+ and Mg^{2+} ions. The results are interpreted in light of the calculated free energy landscapes.

2440-Pos
The Role of Bulges and Hinges in RNA Folding
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Long-range tertiary interactions govern RNA structural assembly, which is a critical step toward RNA biological functionality. Thereby, a universal strategy has emerged for global conformational change; flexible junctions enable unpaired nucleotides to act as beacons between helical regions. Bulges, for example, are versatile secondary structural elements implicated in helix recognition and packing. The P4-P6 domain of the *Tetrahymena* ribozyme utilizes this folding strategy by hinging to form two inter-helical tertiary contacts, the adenine-rich (A-rich) bulge and the tetraloop-tetraloop receptor interactions. To explore the kinetic and thermodynamic properties of tertiary contact formation, we probe the P4-P6 domain hinging and ribose zippering that forms the A-rich bulge interaction using single-molecule FRET methods. We obtain the docking and undocking rates of the A-rich bulge and P4 helix as function of cation concentration and temperature. Docking is accelerated and undocking decelerated by Mg^{2+}. In spite of rapid docking at high $[Mg^{2+}]$ ($k_{dock} = 20 \pm 2\ s^{-1}$), the A-rich bulge interaction is only marginally stable ($K_{dock} = 1.2 \pm 0.1$). These results support that the role of the A-rich bulge is to kinetically direct P4-P6 domain folding while thermodynamic stability is added through the tetraloop-receptor interaction. Formation of the A-rich bulge contact shows specificity for divalent cations, with a preference for Mg^{2+} as anticipated from the Mg^{2+} coordination observed in structural data. A significant kinetic heterogeneity is characterized; only 50% of the molecules exhibit efficient folding at high $[Mg^{2+}]$. Mutations of the A-rich bulge construct reveal a crucial role of the P4-P6 secondary architecture in enabling the A-rich bulge contact.

2441-Pos
Single Molecule FRET Studies of RNA Tertiary Folding: Elucidating the Thermodynamic and Kinetic Role of Na+ Cations
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Understanding the kinetics and thermodynamics of the stabilizing interactions in non-coding RNA provides crucial information about their structural dynamics and ultimately, their biological function. The use of single-molecule FRET methods allows us to investigate the real-time folding and unfolding of an isolated GAAA tetraloop-receptor interaction in the *Tetrahymena* ribozyme. Cation-dependent folding studies of this ubiquitous interaction show that increasing concentrations of Na^+ significantly increase the rate constant for docking and slightly decrease the rate constant for undocking. We examine the temperature-dependence of this Na^+-induced folding in order to determine the thermodynamic parameters associated with the folding and unfolding processes. At 150 mM Na^+ the folding process is exothermic ($\Delta H^\circ = -20$ kcal/mol) but with a significant entropic cost ($\Delta S^\circ = -67$ cal/mol K), leading to a near zero ΔG° at 298 K. Increasing concentrations of Na^+ dramatically increase both ΔH° and ΔS°, with the competition yielding a decrease in ΔG°. For example, by 600 mM Na^+ the folding process even becomes endothermic ($\Delta H^\circ = 4.3$ kcal/mol) and entropically rewarding ($\Delta S^\circ = 20$ cal/mol K), with the folding process now becoming slightly favorable ($\Delta G^\circ = -1.7$ kcal/mol). These results indicate that increasing Na^+ concentration favors folding by increasing entropic gains more than enthalpic losses, which leads to a more favorable folding free energy change. We propose a model that uses the competing roles of solvent and structure to explain these gains and losses.

2442-Pos
The Role of Helix Topology and Counterion Distributions in RNA Interactions
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RNA and DNA helices have the same charge, −2e/bp, but different helical structures. The 2'-OH present in RNA hinders duplex flexibility and promotes the A-form helix whereas the more malleable and polymorphic DNA duplexes prefer the B-form. Using a combination of experimental and computational approaches, we show that the topology of the A-form helix alters the spatial distribution of counterions and is essential in promoting the charge screening efficiency of RNA helices. Results from Anomalous Small-Angle X-ray Scattering (ASAXS) experiments suggest that monovalent and divalent cations are more closely localized to the RNA central axis due to A-form major groove penetration. This leads to very efficient change screening in RNA helices which has implications for ion-mediated RNA interactions in two important areas: RNA folding reactions and the design of short RNA helices for RNA interference applications.

2443-Pos
Temperature-Dependent Single-Molecule Fluorescence Measurements of RNA Motifs Melting
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Single-molecule studies have proven to be a powerful technique for studying folding and unfolding of molecules like proteins and nucleic acids. Population histograms of the Forster Resonance Energy Transfer (FRET) values of double-labeled molecules allow us to discern subpopulations within the ensemble which were previously inaccessible in bulk experiments. Typically, changing solution conditions like pH and ionic strength populate different conformational states allowing the kinetics of folding to be measured. However, temperature as a parameter has not been explored in single-molecule folding studies due to instrument limitations. We have developed and characterized a temperature-controlled single-molecule experimental setup with a range of up to 65C. We used our setup to measure the temperature-dependent melting of nucleic acids motifs like junctions and loops.